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Evaluation of Different Wheat Varieties by SDS-PAGE Electrophoresis

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Abstract: Wheat seed-storage proteins represent not only an important source of food and energy but it is also involved in the determination of bread-making quality. Wheat grains of thirteen wheat varieties were collected from different ecological regions of Pakistan. The variability of seed storage-proteins was analyzed by using SDS-PAGE electrophoresis. Electrophorogram for each variety was scored and presence or absence of each band noted and entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's Similarity Index (JSI) was calculated. Genetic diversity of wheat was evaluated by constructing the dendrogram for High Molecular Weight (HMW) and Low Molecular Weight (LMW) gluten subunit bands. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development especially in developing countries like Pakistan.

Key words: Wheat varieties, SDS-PAGE, genetic diversity, cluster analysis

INTRODUCTION

Wheat (*Triticum aestivum* L.) seed-storage proteins represent an important source of food and energy, being also involved in the determination of bread-making quality (Cooke and Law, 1998). Wheat varieties are qualified to different classes, which exhibit different applications and differ in quantity and quality of proteins, mainly gluten. Gluten, comprising roughly 78 to 85% of total wheat endosperm protein, is a very large complex composed mainly of polymeric (multiple polypeptide chains) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (MacRitchie, 1994). Glutenins confer elasticity to dough, whereas gliadins are viscous and give extensibility to dough (Payne *at al*., 1984).

The pioneer studies of Bietz and Wall (1972) showed that two types of subunits were present, the low molecular weight (10,000-70,000 Da) and the high molecular weight glutenin subunits (80, 000-130,000 Da). High Molecular Weight-Gluten Subunits (HMW-GS) are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (*Glu-A1*, *Glu-B1* and *Glu-D1*) (Payne *et al*., 1980). Electrophoretic studies have revealed appreciable polymorphism in the number and mobility of HMW-GS in both read wheat (Lawrence and Shepherd, 1980; Payne *et al*., 1980) and pasta wheat (Branlard *et al.,* 1989). Bread wheat could, in theory, contain six different HMW-GS but due to the silencing of some of these genes, most common wheat cultivars possess three to five HMW-GS. The Low Molecular Weight-Gluten Subunits (LMW-GS) represents about one-third of the total seed protein and 60% of total gluten (Bietz and Wall, 1973). Despite their abundance, they have received much less research attention than the HMW-GS. This has been mainly due to the difficulty in identifying them in one-dimensional SDS-PAGE gels. The LMW-GS are controlled by genes at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS and 1DS, respectively. Gliadins are heterogeneous mixtures of single-chained polypeptides, molecular weight range is 30, 000 to 75,000 Da. Due to extensive polymorphism, these proteins have been widely used for cultivar identification in hexaploid and tetraploid wheat (Payne *et al*., 1982). Allelic variants of the blocks differ in the number, mobility and intensity of their components and can be characterized through A-PAGE or even SDS-PAGE.

Allelic variation of High Molecular Weight (HMW) subunits of glutenin in 185 cultivars of bread wheat has been described by Payne *et al*. (1981), where about 20

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different major subunits were distinguished by SDS-PAGE. The High Molecular Weight (HMW) glutenin subunits from seven Pakistani wheat genotypes were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), in order to characterize the plant material and test the variability within species (Khan *et al*., 2002). Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits and molecular markers (seed storage proteins separated using SDS-PAGE and DNA markers) (Fufa *et al*., 2005). However a comprehensive study is absent to evaluate different wheat varieties from different ecological region of Pakistan. The present study was undertaken to evaluate the genetic diversity and to ensure the variation in gluten-subunits in set of thirteen Pakistani wheat varieties using SDS-PAGE.

MATERIALS AND METHODS

Plant sample: Wheat Grains of thirteen varieties were collected from different ecological regions of Pakistan during August to December 2005. The samples were stored in labeled glass bottle to ensure safety. The analysis was carried out at the Department of Biotechnology University of Malakand.

SDS-PAGE electrophoresis: The variability of seed storage-proteins was analyzed by using SDS-PAGE (Damania *et al*., 1983) to investigate genetic diversity and characterized wheat varieties. The grains were ground to fine powder and 10 mg was weighed in 1.5 mL microtube, 400 μL protein extraction buffer (Tris-HCL 0.05 M (pH 8), 0.02% SDS, 30.3% Urea, 1% 2-mercaptoethanol) was added to each microtube, kept overnight at 40°C and centrifuged at 13000 rpm for 10 min. The supernatant contain dissolved extracted protein ready for experiment purposes, which could be kept for longer time at 4°C.

Preparation of resolving gel (10% *Acrylamide gel*): The separating gel was prepared by mixing 3 mL (1.875M Tris-HCL Ph 8.80), 6.9 mL distilled water, 5 mL (5% acrylamide), 140 μL (SDS 10%), 90 μL (APS 5%) and 14 μL TEMED.

Preparation of stacking gel: Mixed 1 mL (0.6M Tris-HCL pH 6.8), 7.2 mL distilled water, 1.66 mL (30% acrylamide), 100 μL (SDS 10%), 80 μL (APS 5%) and 9 μL (TEMED) at the last.

Gel preparation: Glass plates were cleaned with 70% ethanol and fixed by using seal gasket and clips, separating gel was poured to the cell and layered with water after 30 min distilled water was removed stacking gel was added. Comb was inserted into the stacking gel.

Sample loading and electrophoresis: Glass cabinet was fixed with electrophoresis apparatus; fill the electrophoretic trays with electrode buffer (25 Mm Tris, 0.1% SDS, 192 mM glycine). Clean the wells with running buffer and load the sample (12 μL) and molecular weight marker 10-200 kDa (Fermentas Protein Ladder) (5 μL) at the bottom of each well using micropipette and connect the power supply at 80 volts.

Staining: After electrophoresis the gel was transferred to tray containing staining solution shake gently for 40 min, followed by destaining until the background of gel disappeared. The picture was taken by gel documentation with white light illuminator.

Data analysis: Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's Similarity Index (JSI) was calculated by the formula (Sneath and Sokal, 1973).

$$S = W/(A+B-W)$$

Where W is the number of bands of common mobility, A the number of bands in type A and B is the number of bands in type B. The similarity matrix generated was converted to a dissimilarity matrix (Dissimilarity = 1-similarity) and used to construct dendrogram by the unweighed pair group method with arithematic means (Sneath and Sokal, 1973). All analysis was carried out using a statistical package NTSYS-PC, version 1.8 (Rohlf, 1993) and STATISTICA for window 98.

RESULTS

Genetic diversity evaluation: In this study SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of Gluten subunits and investigate genetic diversity among different Pakistani wheat varieties. Electrophorogram showing proteins banding pattern of different wheat varieties are given in Fig. 1 and 2. A total of 21 bands were obtained among which bands number 1, 2, 4, 7, 12, 13 and 20 were common in all varieties but the other bands shows variation. The results from comparison with standard molecular weight marker reveal that wheat variety Tatara, Bakhtawar-92, Bhakkar-01 and Gaznawy contain 7 subunits in range of 50-120 kDa while Fakhr-e-Sarhad and Zakht represent 11 and 9

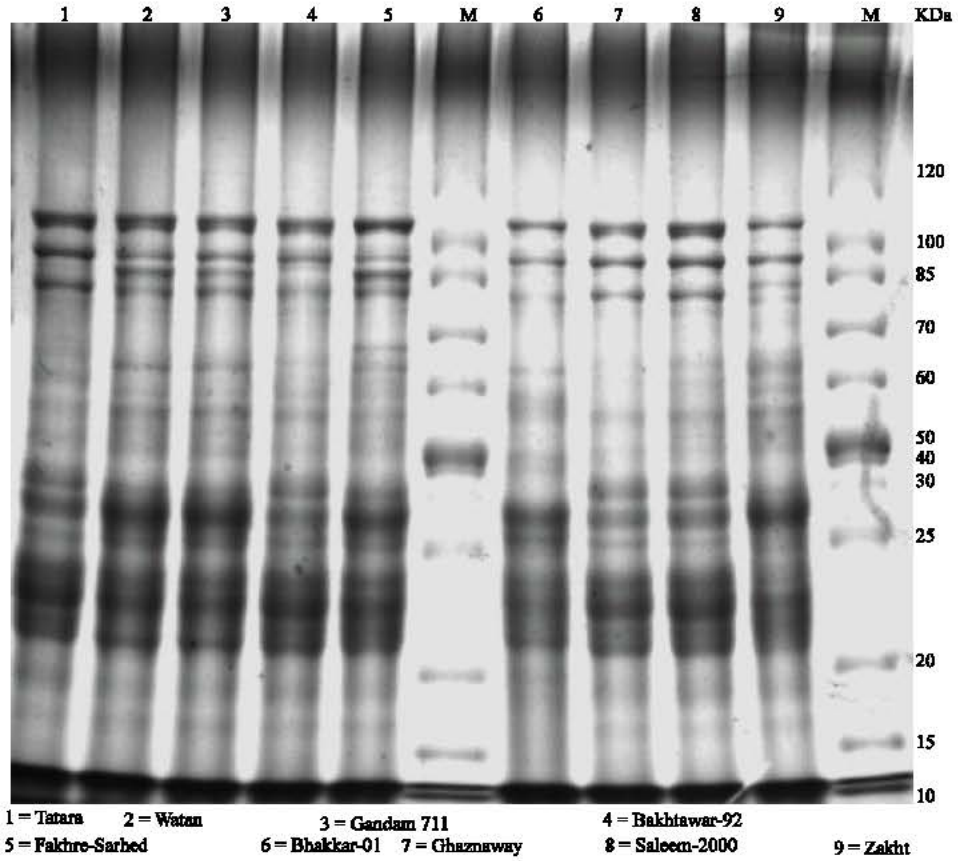

Fig. 1: Electrophorogram showing banding pattern of wheat proteins and molecular weight marker

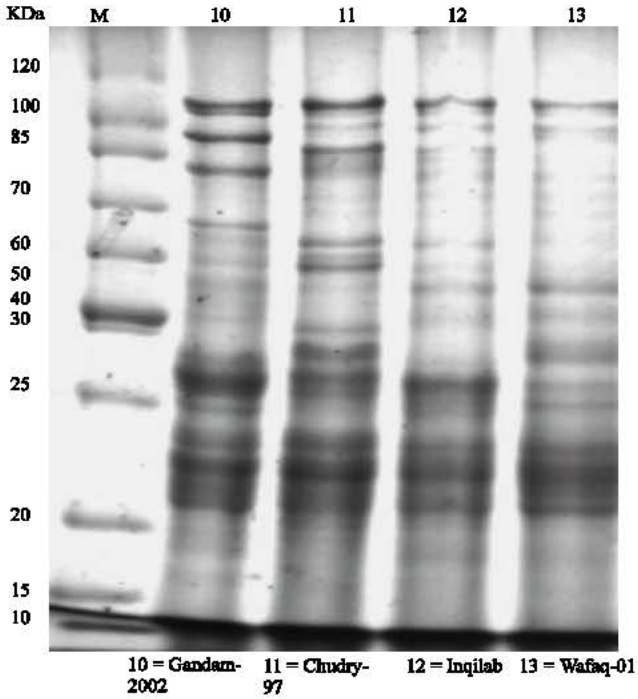

Fig. 2: Electrophorogram showing banding pattern of wheat proteins and molecular weight marker

subunits respectively. At low molecular weight range of 15-50 kDa there are 6-12 subunits reflecting less diversity but variety Chudry-97 and Wafaq-01 showing more variation than the rest of varieties. The varieties Watan, Gandam 711, Fakhre-Sarhad, Zakht and Chudry-97 appear to contain 85-kDa gluten, while other contains none of these. The study has thus revealed some striking variations among the wheat varieties regarding their genetic diversity. Further investigation may be helpful in classification of the cultivars into groups containing similar varieties.

Cluster analysis on the bases of SDS-PAGE: Cluster analysis of wheat grain storage proteins was performed on the results of SDS-PAGE using the software STATISTICA to find out the diversity among the given wheat varieties. The results of cluster analysis are given in the dendrogram (Fig. 3) on the bases of linkage distance (Euclidean distances) Table 1. The diagram revealed two main groups L_1 and L_2; the group L_1 has only one variety Wafaq-01 zand L_2 comprised the remaining 12

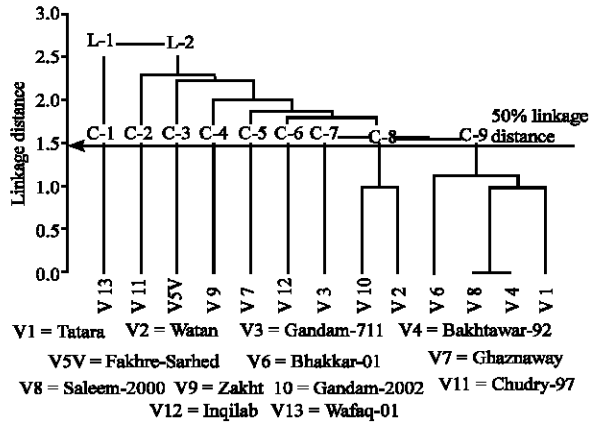

Fig. 3: Dendrogram of thirteen wheat varieties based on SDS-PAGE (UPGMA)

wheat varieties. At Euclidean distance of 2.5 all the varieties show similarity with one another and distributed into two categories one containing the variety chudhry-97 while the second is further divided into subgroups in which one include only one variety Fakhre-Sarhad. At linkage distance 2 the Zakht variety show more than 50% distance with the rest of the varieties. Below the linkage distance of 1.5 only the variety Gandam-2002 and Watan are in one group and Saleem-2000, Bhakkar-01, Bakhtawar-92 and Tatara are in the other group showing less than 50% linkage distance. The varieties Saleem-200 and Bakhtawar-92 show 100% similarity.

DISCUSSION

According to the results of SDS-PAGE, the variability in HMW-GS is present but the overall pattern of seed storage-proteins shows low degree of heterogeneity. The diversity in high molecular weight protein subunits is the result of gene silencing in some varieties encoding these proteins (Lawrence and shephred, 1980). SDS-PAGE electrophoresis of seven wheat varieties has been investigated including Inqilab-91 for HMW gliadin, however their varieties were different but the final result is correlated (Khan *et al*., 2002). Together with physicochemical and molecular characteristics already reported (Khan *et al*., 2006; Zeb *et al*., 2006) this study present a good tool to characterize seed storage protein as such in achieving the planned objective of the research.

Cluster analysis on the bases of SDS-PAGE: The dendrogram calculated from the Jaccard similarity coefficient and un-weighted pair group method with averages constructed by HMW and LMW glutenin subunit bands. Genetic diversity of European spelts wheat was evaluated by constructing the dendrogram for HMW and LMW gluten subunit bands

Table 1: Molecular weight analysis of wheat varieties

Protein type	Molecular weight (kDa)	Wheat variety 1	2	3	4	5	6	7	8	9	10	11	12	13
HMW- GS	120	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0
	85	0	1	1	0	1	0	0	0	1	0	1	0	0
	70	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	1	1	1	1	1	1	1	1	1	1	1	1	0
LMW- GS	50	1	1	1	1	1	1	1	1	1	1	1	1	1
	40	0	0	0	0	0	0	0	0	0	0	1	0	0
	30	1	1	1	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	1	1	1	1	1	1	1	1	1	1	1	1	1
	15	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0

V1 = Tatara, V2 = Watan, V3 = Gandam 711, V4 = Bakhtawar-92, V5V = Fakhre-Sarhad, V6 = Bhakkar-01, V7 = Ghaznawy, V8 = Saleem-2000, V9 = Zakht, V10 = Gandam-2002 V11= Chudry-97, V12= Inqilab-91 V13=Wafaq-01

(Xueli *et al*., 2005). The results of cluster analysis are given in the dendrogram (Fig. 3) on the bases of linkage distance (Euclidean distances). The figure revealed two main groups L_1 and L_2; the group L_1 has only one variety Wafaq-01 and L_2 comprised the remaining 12 wheat varieties. At Euclidean distance of 2.5 all the varieties show similarity with one another and distributed into two categories one containing the variety chudhry-97 while the second is further divided into subgroups in which one include only one variety Fakhr-e-Sarhad. At linkage distance 2 the Zakht variety show more than 50% distance with the rest of the varieties. Below the linkage distance of 1.5 only the variety Gandam-2002 and Watan are in one group and Saleem-2000, Bhakkar-01, Bakhtawar-92 and Tatara are in the other group showing less than 50% linkage distance. The dendrogram as a whole revealed low genetic diversity at proteins level because most varieties are in the same cluster. Fufa *et al*. (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait. The variety Saleem-2000 and Bakhtawar-92 show 100% similarity with one another representing the duplication of the variety with different names in different resource laboratories. Further investigation of this research may reveal more duplication of varieties.

From the results of this study it is therefore concluded that seed storage protein profiles could be useful markers in cultivar identification, registration of new varieties, pedigree analysis and in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development especially in developing countries like Pakistan. The method could be applied as a pre-registration requirement for the preliminary assessment and evaluation of duplication in proposed cultivars.

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